

Synthesis and Characterization of Janus Fenugreek Seed Gum-Based Film for Food Packaging and Wound Dressing Applications

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Abstract

The use of plant-derived natural gum polysaccharides in food packaging and biomedicine is growing due to their biocompatibility, biodegradability, film-forming ability, abundant functional groups, and potential for chemical or physical modification to develop novel materials. In this study, fenugreek (*Trigonella foenum-graecum*) seed gum (FSG)-based Janus films with glycerol (GLY) as a plasticizer in varying concentrations (2%, 5%, 7%, and 10% w/w) were synthesized for potential food packaging and biomedical applications. The glycerol concentration significantly affected the stability, microstructure, moisture content, and solubility of the film, with 5% GLY yielding the most stable films. The water solubility was gradually increased with increasing the GLY content in the film sample. The film exhibited strong radical scavenging activity against DPPH assays, with an IC₅₀ value of 158.73±0.013 µg/mL. The total phenolic content (TPC) and total flavonoid content (TFC) of the film were found to be 88.21±0.012 mg/g in gallic acid equivalents (GAE) and 9.36±0.018 mg/g in quercetin equivalents (QE), respectively. The pristine FSG film exhibited antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and the fungal strain *Candida albicans*, which was significantly enhanced by incorporating the antibacterial drug penicillin. Additionally, we examined the impact of different films on chicken meat and cheese by monitoring changes in weight loss, color, pH, total aerobic mesophilic counts (TAMC), and psychrotrophic bacterial counts (PBC) during storage. Our results demonstrated that the samples packaged with pristine FSG film exhibited significantly lower TAMC and PBC values and slowed down the increase in pH values compared to the control samples, which further decreased by incorporating antimicrobial drug. These findings indicated that the Janus pristine FSG film is suitable for food packaging. Further, antimicrobial drugs incorporated into FSG film could be potential candidates for wound dressing applications.

Keywords: Fenugreek; Antimicrobial film; Total aerobic mesophilic count; Psychrotrophic bacteria count

Introduction

The skin is a delicate organ that protects the

body from the external environment by forming an interface between the external environment

and internal milieu and protects from uncontrolled water loss and numerous environmental stressors, including mechanical injury, UV irradiation, thermal and chemical reagents, harmful microorganisms, and viruses [1, 2]. Skin injury is painful, and recovery is a very complex phenomenon involving protection from microorganisms, cell migration, and tissue regeneration [3]. A small injury to the epidermal layer is usually cured by a process called reepithelization, whereas for larger wounds, like damage to the dermal layers, the body system cannot heal itself or delays the wound healing process. Further such injuries can result in wound infections, which in turn can lead to postoperative complications, necessitate frequent hospital visits and prolonged hospital stays, and increase both direct and indirect healthcare costs associated with treating these infections [4]. In such a case, wound dressing is a common procedure to maintain a moist environment, protect against bacterial infection, support cell adhesion and proliferation, and enhance the wound healing process [5]. Biopolymers are gaining prominence in wound dressing applications because of their compatibility with biological tissues, their ability to break down naturally, and their effectiveness in supporting the healing process [6]. Chitosan, alginate, and hyaluronic acid and their composites are commonly used for their inherent antimicrobial properties, moisture retention, and ability to facilitate cell proliferation and tissue regeneration [7]. These natural polymers offer a favorable environment for wound healing by maintaining a moist wound interface, which is crucial for optimal healing. Additionally, biopolymers can be engineered to deliver drugs or growth factors directly to the wound site, further enhancing their therapeutic potential [8]. Their versatility

and functional properties make biopolymers an attractive alternative to traditional synthetic wound dressings in both clinical and home care settings. Despite their advantages, the extraction of these polymers requires extensive processes, making them less cost-effective. Therefore, a novel biosources for wound dressing is crucial.

Fenugreek (*Trigonella foenum-graecum* L.), a leguminous plant in the Fabaceae family, is well known for its fiber, gum, other volatile contents, and chemical constituents [9]. This plant is extensively cultivated as a semiarid crop in Northern Africa, the Mediterranean, Nepal, India, and Canada. It exhibits a range of pharmacological properties, including hypoglycemic, anticancer, anti-inflammatory, antioxidant, and antibacterial effects [10]. The seeds and green leaves of fenugreek are used in food as well as in medical applications, which is an old practice in human history [11]. Fenugreek seed gum (FSG) is a natural polymer extracted from the endosperm of fenugreek seeds with a galactomannan-like structure composed of galactose and mannose [12]. These compounds have several crosslinking sites and can form films with tunable physical and mechanical properties with different crosslinkers [13]. Furthermore, FSG has been widely used as an antioxidant and anti-fungal agent in food systems due to its high antioxidant and antimicrobial activities [14]. Furthermore, FSG has an excellent emulsification capacity, which enables its application in the food, cosmetic, and/or pharmaceutical industries [15]. FSG is abundant in functional groups, offers multiple sites for highly selective coupling reactions, and can be chemically or physically modified to create new materials. These functional groups could be effective for binding various types of drugs for biomedical

applications [16]. While fenugreek gum is commonly used as a food additive, its application as a food packaging and biomedical material remains limited. The FSG-based film with high antioxidant and antimicrobial activity could be a potential candidate for food packaging and wound dressing applications.

In this study, FSG-based films were prepared using glycerol as a plasticizer. The effects of glycerol concentrations on the physical and chemical properties of films were thoroughly investigated for food packaging applications. Furthermore, antimicrobial drugs penicillin was incorporated into the FSG film, and the antimicrobial activity was investigated against Gram-positive and Gram-negative bacteria. The antimicrobial activities were investigated through microbiological and physicochemical analyses of chicken and cheese samples packaged in FSG based films.

Materials and Methods

The fenugreek seeds were purchased from the local market of Kathmandu, Nepal.

Preparation of film

10 g of fenugreek seeds (FSG) were washed with ethanol, dried at about 70 °C for 10 minutes, and then soaked in 100 ml of miliQ water for 10–15 hours at 35 °C. Later, the seeds were crushed and filtered with Teflon cloths to obtain FSG. Different amount of glycerol (2%, 5%, 7%, and 10% w/w) was added to the solution and centrifuged for 10 minutes at 5000 rpm. Which was cast on a petri plate to develop the film. For the preparation of antimicrobial films, penicillin solution (1mg/mL) was prepared in warm water, and 1 mL of each solution was mixed separately, with the prepared samples having different concentrations of glycerol. The mixtures were homogenized for 1 minute and poured into clean, sterile petri plates. The films were dried at 35 °C

for 2 days and stored in a desiccator for further analysis.

Determination of the thickness of the film

The film thickness was determined by a digital micrometer (Mitutoyo Corp. MDC-1 SB) with a precision of 0.01 mm. >10 random locations of the film were measured to compute the average film thickness.

Determination of water solubility

The film samples of 2×2 cm² were taken and dried in an oven at 85 °C for 24 hours. The initial solid content of the samples (W₀) was measured. These dry film samples were immersed in 30 mL of miliQ water for 24 hours at 25 °C in an incubator. The water was discarded, and the soaked film samples were dried at 85 °C for another 24 hours. Then, the final weight (W_f) of dry films was taken. The water solubility of film was calculated using equation (1) [17].

$$\text{Solubility} = \frac{W_0 - W_f}{W_0} \times 100 \quad (1)$$

Determination of the total phenolic and total flavonoid content

The total phenolic content was analyzed by the Folin-Ciocalteu colorimetric method based on an oxidation-reduction reaction. Gallic acid was used as a standard [18]. The total flavonoid content was determined by an aluminum chloride colorimetric assay using Quercetin as a reference [19].

Determination of the antioxidant properties of the film

The antioxidant activity of extracts was assessed by DPPH free radical scavenging activity. Ascorbic acid was used as a standard solution. The free radical was determined using equation (2):

$$\text{Scavenging activity} = \frac{(A_0 - A)}{A_0} \times 100\% \quad (2)$$

Where A₀ is the absorbance of the reference (blank solution without extract), A is the absorbance of the sample extract. The

calibration curve was drawn taking sample concentration as x-axis and percentage radical scavenging activity as y-axis for both ascorbic acid and sample solution. IC₅₀ values were calculated using the inhibition curve.

Determination of antimicrobial efficacy of the films

The antimicrobial susceptibility test was carried out by the disk diffusion method. Disk size of 6mm were used for the evaluation of the potential antimicrobial activity of the films. The inoculums of bacteria and fungi were transferred into petri dish containing solid media of agar using sterile swab. Inhibition of microbial growth was measured in the form of a zone of inhibition (ZOI) after incubation at 37°C for 24 hours in bacteria and at 25°C for 48 hours in fungi.

Food spoilage prevention test

Chicken and cheese samples (20–25 g) were wrapped in different films and stored at 4 °C for up to 5 days. The overall quality of samples was evaluated by microbiological and physicochemical analysis at 0, 3, and 5 days of storage.

Microbiological analyses of chicken meat and cheese

Chicken breast meat samples and cheese samples of 1 g each were homogenized in 9 ml of sterile 0.1% peptone water. Serial dilutions (1:10) were prepared in a 0.1% peptone water solution, and appropriate dilutions of homogenates were taken for the analysis. Total aerobic mesophilic counts (TAMC) were determined by taking a dilution of 10⁻³. About 100 µL of homogenate was taken and spread onto the PCA plates, which were incubated at 30 °C for 48 hours. Psychrotrophic bacteria counts (PBC) were determined as previously, except that they were incubated at 4 °C for 7 days. Microbiological counts were expressed as logarithms of the number of colony-forming

units per gram of samples (log cfu/g). Microbiological analyses were carried out in duplicate during storage [21].

Determination of weight loss and pH of the samples

The weight loss was calculated as a percentage loss of initial weight. All physicochemical measurements were performed in triplicate. 10 g of chicken sample and 10 g of cheese sample were homogenized with 100 ml of distilled water for 1 minute, and the pH values of the homogenates were measured using a pH meter

Results and Discussion

Physical characterization of the film

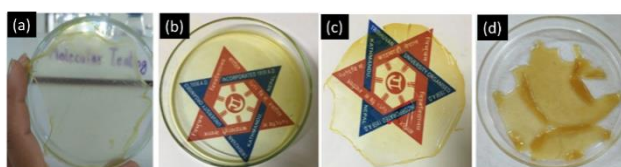


Figure 1: FSG film with different concentrations of glycerol: (a) 2% GLY, (b) 5 % GLY, (c) 7% GLY, and (d) 10% GLY

The gum was extracted from the fenugreek seeds and used to prepare the bioplastic films. The varying amount of glycerol was blended with FSG, centrifuged for 10 minutes at 500 rpm, and cast to synthesize the biodegradable film. **Fig. 1** shows the digital images of the bioplastic films prepared by varying the concentrations of the glycerol (2%, 5%, 7%, and 10% w/w) with respect to FSG. The bioplastic film synthesized with 2% glycerol (w/w) (**Fig. 1(a)**) was transparent, rigid, brittle, making it difficult to handle and remove from the Petri dish. Increasing the glycerol concentration to 5% significantly improved the flexibility of the film. However, raising the glycerol concentration to 10% caused the bioplastics to turn yellow and appear excessively oily, wet, and sticky (**Fig. 1(d)**). The bioplastics containing 5% and 7% glycerol (w/w) were transparent, easy to handle,

and had a yellowish hue (**Fig. 1(b) and 1(c)**). It can be seen that the film with 5% glycerol exhibited very smooth, compatible morphologies with no cavities, edges, or holes. This indicates strong chemical interactions between FSG and glycerol. The results indicated that an optimal glycerol concentration of 5-7% (w/w) facilitated the formation of a homogenous FSG matrix. This concentration effectively promoted plasticization, resulting in a single-phase morphology without any separation. The sticky nature of bioplastics at higher concentrations of glycerol might be attributed to phase separation and glycerol diffusion at the surface of the film.

The thickness of the for 2% GLY film was 0.07 ± 0.02 mm, which was increased to 0.08 ± 0.08 mm, 0.09 ± 0.06 mm, and 0.13 ± 0.03 mm for 5 % GLY film, 7% GLY film and 10% GLY film, respectively. This result revealed that the film thickness increased with increasing the GLY concentration, which could mainly be related to the more significant amount of solid content in the film-forming solutions.

Antioxidant properties

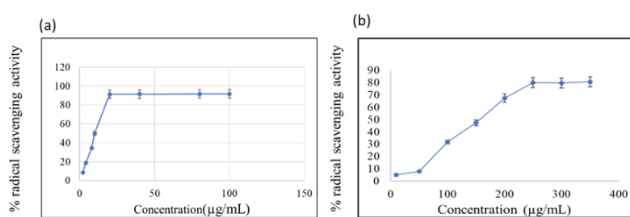


Figure 2: (a) graph depicting DPPH radical scavenging activity as a function of ascorbic acid concentration, (b) graph showing % radical scavenging activity vs. concentration of FSG film.

The antioxidant properties of the bioplastic film are very important for food packaging and tissue engineering applications [21]. **Fig. 2** shows the scavenging activity as a function of ascorbic acid concentration and the radical scavenging activity vs. the concentration of FSG film. The antioxidant activity of the FSG film was evaluated using the DPPH assay, and an IC_{50} value was found to be 158.73 ± 0.01 $\mu\text{g/mL}$. This

result confirms that FSG films have potent antioxidant properties. The antioxidant properties depend up on the total phenolic content and the flavonoid content of the scaffold. The total phenolic content of the synthesized FSG film was found to be 88.21 ± 0.01 mg/g GAE, which agrees with the previous report [22]. The total flavonoid present in the methanolic extract of FSG film was estimated by the aluminum chloride colorimetric assay using quercetin as a standard and found to be 9.36 ± 0.01 mg/g QE. Previous studies also reported that the total flavonoid content of fenugreek seeds ranges from 4.75 to 11.21 mg/g across different varieties [23]. Due to high phenolic and flavonoid content, fenugreek seeds are regarded as one of the richest sources of natural antioxidants.

Antibacterial and antifungal activity

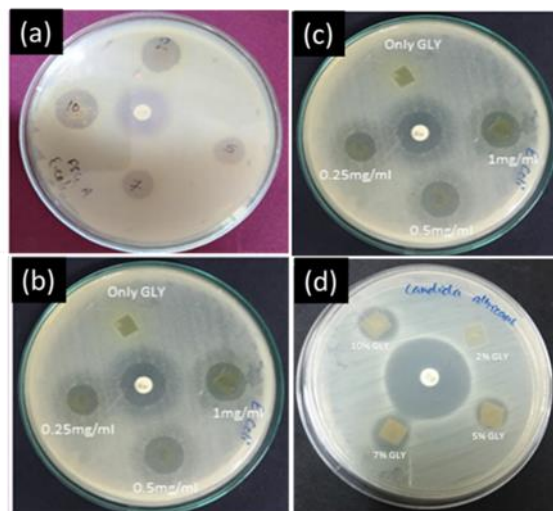


Figure 3: Zone of inhibition of different films. (a) Pristine FSG film with different concentrations of GLY (2%, 5%, 7%, and 10% w/w) against *E. coli*, (b) FSG film with different concentrations of penicillin against *E. coli* (c) FSG films with different concentrations of penicillin against *S. aureus*, (d) Pristine FSG film with different concentrations of GLY (2%, 5%, 7%, and 10% w/w) against fungal strain *Candida albicans*

FSG film with a 5% glycerol concentration was found to be mechanically flexible and stable

in water. This formulation was used to incorporate the model antimicrobial drug penicillin for wound dressing application. As-prepared films with and without drugs were screened for their antimicrobial activity against a gram-positive bacterium, *S. aureus*, and a gram-negative bacterium, *E. coli* via the zone of inhibition method. **Fig 3(a)** shows the antibacterial activity of FSG films with different concentration of GLY. It can be seen that the pristine FSG film itself has antibacterial activity, which is attributed to the protein in FG seeds [24]. Incorporation of the antibiotic drug significantly enhanced the zone of inhibition for both bacterial strains, which was gradually increased along with the concentration of the drug in the film, as shown in **Fig. 3(b) and 3(c)**. These results revealed that the antimicrobial drug penicillin has a good interaction with the FSG matrix and can be effectively incorporated in different amounts for various applications. Furthermore, the fungal growth inhibitory activity of pristine FSG with different concentration of GLY were studied against *Candida albicans* and the result is shown in Fig. 3(d). It can be seen that all the samples are equally effective against fungal strain. These results suggested fenugreek seed itself has antimicrobial and antifungal properties and could be used for food packaging applications.

Solubility and biodegradation of film

Water solubility is a crucial parameter for the application of bioplastics. The solubility of FSG films with varying glycerol concentrations was examined, revealing that films containing 2%, 5%, 7%, and 10% glycerol had solubilities of 24.36 ± 0.01 , 36.46 ± 0.01 , 49.28 ± 0.02 , and 58.04 ± 0.01 , respectively. These findings indicate that film solubility increases with higher glycerol concentrations. This trend is attributed to the increase in hydroxyl (OH)

groups within the FSG film matrix as glycerol concentration rises, enhancing the films' interactions with water molecules. A FSG film (5% GLY) was tested for biodegradation, showing 75% degradation in 7 days as shown in **Table 1**. This indicates high biodegradability of fenugreek seed gum bioplastic, suitable for wound dressing and short-shelf-life food packaging like vegetables, meat, and dairy. Solubility test supports biodegradation findings.

Table 1: Degradability test of fenugreek seed gum (FSG) film

Films	Initial	Final weight (g)			
	Weight(g)	Day1	Day3	Day5	Day7
FSG	1	0.787	0.543	0.371	

Physicochemical analysis FSG film coated

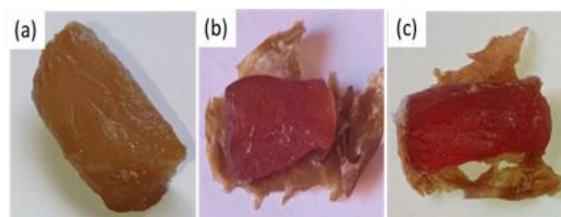


Figure 4: Chicken meat samples after one week at ambient conditions. (a) Sample without packaging, (b) Sample Packaged with FSG pristine film, and (c) Sample packaged with FSG-P film.

Microbial growth in meat can result in slime formation, structural component degradation, off-odor, texture, and appearance change [25]. The meat samples were wrapped in FSG and penicillin incorporated FSG film (FSG-P) films for one week at 25°C, and compared with control samples. The control sample (bare sample without packaging) showed a significant change in color and texture (see Fig. 4(a)). It was giving off an odor, revealing the deterioration in its quality. Meat samples packaged in pristine FSG films (see **Fig. 4(b)**) had minor changes in color and texture, but no deterioration in quality was observed. Meat samples packaged with FSG-P

films (see **Fig. 4(c)**) were observed to be fresh and dry without changes in their color or texture. This observation suggests that pristine FSG films are suitable for food packaging, extending shelf life without compromising quality. Additionally, drug-incorporated films can be utilized in wound dressings to protect the skin from antimicrobial infections.

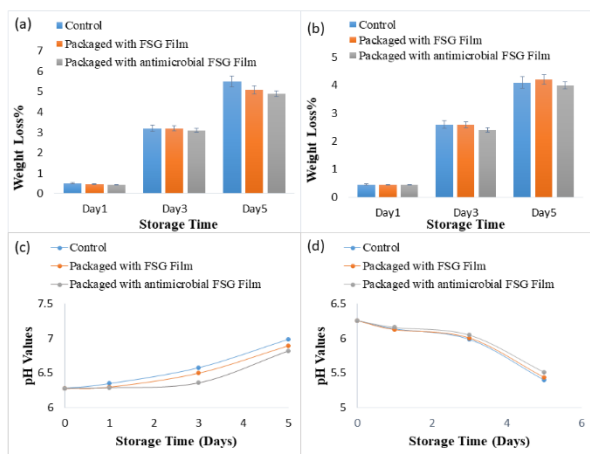


Figure 5: Weight loss percentage of (a) chicken meat samples, (b) cheese samples. pH values at different time intervals (c) chicken meat samples, and (d) cheese samples.

Fig. 5 shows the weight loss of the different meat and cheese samples for different time intervals. It can be seen that the weight loss increased for all the samples with an increase in the storage time interval. No significant difference in weight loss was observed among samples until the three-day interval. However, on the 5th day, bare samples exhibited slightly higher weight loss compared to packaged samples. Meat samples wrapped with FSG-P exhibited the lowest weight loss compared to bare and FSG-wrapped samples (**Fig. 5(a)**). Furthermore, we monitored the change in pH of the different samples during 5 days of storage, as depicted in **Fig. 5(c) and 5(d)**. The initial pH of fresh chicken meat was 6.28 ± 0.03 , and the pH values of the samples slightly changed

during 5 days of storage for both control and packaged samples. However, there was a slight increase in the pH values of each meat sample. The packaged samples showed lower pH values than in the control samples, which can be attributed to the antioxidant and antimicrobial properties of the film. A major reason for the increase in pH of chicken meat may be the accumulation of metabolites from microbial growth, such as amines and ammonia, produced by psychotropic bacteria [26]. A similar trend was observed with cheese samples packaged with various FSG films (**Fig. 5(d)**), showing only slight changes in the pH values. As cheese is an acid-forming food, its pH value remained below neutral, with no significant difference among the samples.

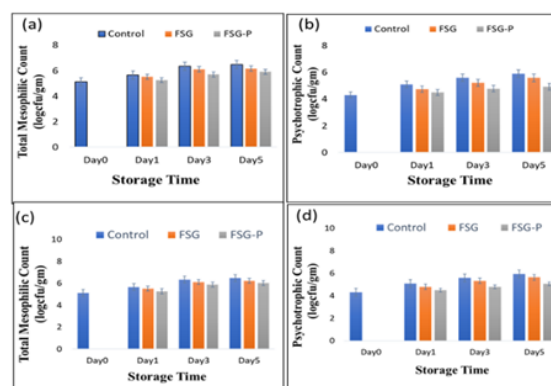


Figure 6: Microbiological analysis of various samples. (a) TAMC for meat samples packaged with FSG and FSG-P film; (b) PBC for meat samples packaged with FSG and FSG-P film. (c) TAMC for cheese samples packaged with FSG and FSG-P film, and (d) PBC for cheese samples packaged with FSG and FSG-P film

The total aerobic mesophilic counts (TAMC) and psychrotrophic bacteria counts (PBC) are important parameters that reveal the efficacy of the film. TAMC and PBC values of chicken breast meats in both treatments increased during storage at 4 °C, as shown in **Fig. 6** and **Fig. 7**. This increase was higher in control samples than in packaged samples. The initial TAMC of chicken breast meat was 4.78 ± 0.40 log cfu/g, which was increased to 6.54 ± 0.078 log

cfu/g on day 5 for the control sample (**Fig. 6a**). The initial TAMC is similar to the previous study [27]. Both packaged samples showed a lower TAMC value compared to the control sample. The TAMC value of fresh cheese bought from the local market was 5.11 ± 0.38 log cfu/gm, which was up to 6.47 ± 0.097 log cfu/gm for the control sample, as shown in **Fig. 6(c)**. Both the packaged samples exhibited lower TAMC values compared to the control sample. However, the samples packaged with FSG film exhibited slightly higher TAMC values than those packaged with FSG-P film. The PBC values for all samples were almost same until day 3, as shown in **Fig. 6(a)**. However, on the 5th day, the control sample exhibited slightly higher PBC compared to packaged samples. As shown in **Fig. 6(b)**, chicken breast meat demonstrated lower psychotropic counts on days 3 and 5. The psychotropic bacteria count (PBC) value for cheese samples also increased in storage from 4.3 ± 0.41 logcfu/gm and exceeded 5.93 ± 0.18 logcfu/gm (**Fig. 6(d)**). These results suggested that the FSG-P film was more effective against total aerobic mesophilic bacteria.

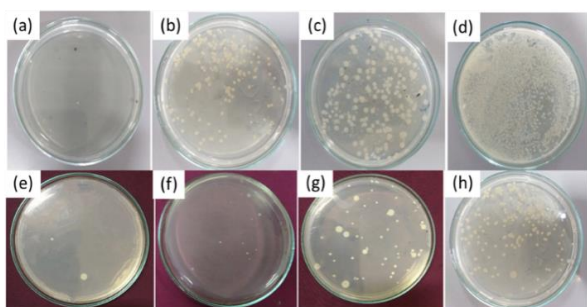


Figure 7: Petri plates showing TAMC of meat sample (a) control at day 0, (b) packaged with FSG-P film at day 5, (c) packaged with pristine FSG film at day 5, (d) control meat sample. Petri plates showing PBC of cheese samples (e) control at Day 0, (f) packaged with FSG-P film at Day 5, (g) packaged with FSG film at Day 5, and (h) control sample at Day 5.

The FTIR analysis was carried out in order to understand the bonding interaction of FSG with

penicillin (**Fig. 8**). The broad peak between 3100 and 3500 cm^{-1} corresponds to the O-H stretching of hydroxyls and bound water. The vibration at 2800–3000 cm^{-1} is assigned to C-H stretching. In addition, all film samples exhibited a sharp peak at 1039 cm^{-1} , which was caused by the vibration of C-O-C bonds. These results suggested that the various functional groups such as carboxyl, hydroxyl, fats, alcohols, amides, and phenolic compounds existed in fenugreek seed gum [28]. The slight changes in characteristic peaks reflect the chemical interactions between the film constituents, namely FSG and glycerol and incorporated penicillin.

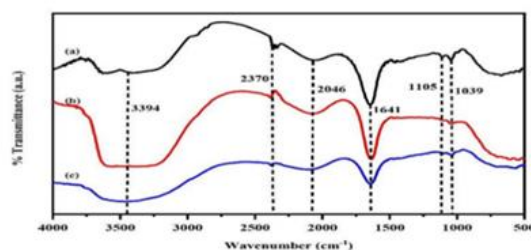


Figure 8. A Graph showing the FTIR spectra of (a) FSG, (b) Pristine FSG film (c) Film incorporated with Penicillin (FSG-P).

Conclusions

Fenugreek seed gum was successfully employed for the preparation of flexible, transparent edible films, varying the concentration of glycerol. Glycerol concentrations of 5–7% (w/w) were found to be successful in preparing high-quality films. FSG films were found to be good natural antioxidants with potent DPPH free radical scavenging properties compared with standard ascorbic acid. FSG films showed a total phenolic content of 88.21 ± 0.021 mg/g gallic acid equivalent and a total flavonoid content of 5.88 ± 0.018 mg/g quercetin equivalent. Pristine FSG films showed antibacterial activity against *E. coli* (gram-negative bacteria) and *S. aureus* (gram-positive bacteria) and against the fungal strain *Candida*

albicans due to peptide groups of protein, which can be enhanced by incorporating drugs. FSG films showed great potential as food packaging materials due to their flexible, mechanical, and biodegradable nature. In the experiment with TAMC and PBC for both the samples, meat and cheese, there was an appreciable difference between the control samples and the samples packaged with pristine FSG films. TAMC, and PBC for control samples were high in comparison to samples packaged with FSG film and antimicrobial FSG films. All these results revealed that the pristine FSG films is a potential candidate for food packaging material. Penicillin incorporated FSG films could be used as antibacterial wound dressing material.

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Author's Contribution Statement

Susmita Bhattarai: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kalpna Khanal:** Methodology, Investigation, Formal analysis, Data curation. **Dikpal Kumar Shahi:** Formal analysis, Methodology. **Rajendra Bahadur GC:** Formal analysis, Methodology. **Narayan Bhattarai:** Writing – review & editing. **Lok Ranjan Bhatt:** Writing – review & editing, Supervision, Validation, Funding acquisition, Methodology. **Mahesh Kumar Joshi:** Writing – review & editing, Supervision, Funding acquisition, Methodology, Conceptualization.

Conflict of Interest

The authors declare no competing financial interest.

Data Availability Statement

The data that support the findings of this study can be made available from the corresponding author, upon reasonable request

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